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EXAMINER
CANELLA, KAREN A

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1642	16

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/430,175	Applicant(s) Lesko et al
	Examiner Karen Canella	Art Unit 1642

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3 months* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is FINAL. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1, 2, 4, 5, 9-25, 28-41, 43-56, and 58-70* is/are pending in the application.

4a) Of the above, claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1, 2, 4, 5, 9-25, 28-41, 43-56, and 58-70* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a)☐ All b)☐ Some* c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

15)☒ Notice of References Cited (PTO-892)

16)☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

17)☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s). 5, 5, 5, 7, 9, 14 ~~5, 7, 9,~~

18)☐ Interview Summary (PTO-413) Paper No(s). _____

19)☐ Notice of Informal Patent Application (PTO-152)

20)☐ Other:

Response to Amendment

1. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office Action.

2. Claims 3, 6, 7, 8, 26, 27, 42, 57 have been canceled. Claims 1, 34-41, 43, 44, 47, 53, 54 have been amended. Claims 59-70 have been added. Claims 1, 2, 4, 5, 9-25, 28-41, 43-56 and 58-70 are under consideration.

New Grounds of Rejection

3. Claims 5, 9, 10 and 54 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

(a) Claim 5 is vague and indefinite as it depends on canceled claim 3. For purpose of examination, claim 5 will be read as depending on claim 4.

(b) The recitations of "surface" in claim 9 and "fixative" in claim 10 lack proper antecedent basis in claim 1. For purpose of examination, the claims will be read as being dependent on claim 2.

(c) Claim 54 recites "Characterizing a single cell environment". It is not clear if the single cell environment is the intracellular or extracellular environment of the single cell. For purpose of examination, both alternatives will be considered.

(d) Claim 54 recites "wherein the concurrent measurement of multiple cellular markers using fluorescent probes," but fails to recite an action that is to be taken after said concurrent measurement. For purpose of examination, "wherein the concurrent" will be read as ---by the concurrent--.

4. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless --

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

5. Claims 1, 2, 4, 5, 9, 10, 11, 12, 33-38, 40, 41, 43-46, 51, 53-56, 59, 62-70 are rejected under 35 U.S.C. 102(a) as being anticipated by Wang et al (International Symposium on Biology of Prostate Growth, March 1998, reference AS18 of the IDS filed 2/15/2002).

Claim 1 is drawn to a method of characterizing single circulating epithelial cancer cells obtained from a body fluid comprising the concurrent measurement of multiple cellular markers using fluorescent probes, wherein said probes emit different wavelength of light to distinguish multiple cellular markers expressed in said single cells using fluorescent microscopy. Claim 2 embodies the method of claim 1, wherein said single cell is isolated by density gradient centrifugation from a sample containing cells, said isolated cells are fixed onto a surface and incubated with the probes of claim 1, wherein probes binding to the cellular markers is examined by fluorescent microscopy for the identification of each specific marker. Claim 4 embodies the method of claim 1 wherein cells are isolated from body fluid by density gradient centrifugation. Claim 56 embodies the method of claim 4, wherein cells are further isolated by a negative selection process. Claim 5 embodies the method of claim 2, wherein said body fluid is selected from a group comprising blood. Claim 9 specifies that the surface for adherence in claim 2 is a microscope slide. Claim 10 specifies various fixatives. Claim 11 embodies the covalent linkage of fluorescent compound to a probe. Claim 12 specifies the selection of fluorescent probes based on minimal overlapping emission spectra, enabling the concurrent use of the probes in said single cell. Claim 33 is drawn to the method of claim 1, wherein the probes comprise multiple fluorescent probes that emit light of different wavelengths with minimal interference between wavelengths of said emitted light when using appropriate filter set combinations that allow one marker to be distinguished from another when tested concurrently. Claim 34 embodies the method of claim 1, wherein said probe is directed to a cellular target which is not a nucleic acid. Claim 35 embodies claim 35, wherein said probe comprises a protein or peptide. Claim 36 embodies claim 35, wherein said probe is an antibody. Claim 37 embodies the method of claim 1, wherein the probe

is a nucleic acid. Claim 38 embodies the method of claim 37, wherein said probe comprises DNA. Claim 40 embodies the method of claim 1 wherein the probes are a combination of probes which are directed to a cellular target which is not a nucleic acid and probes which are a nucleic acid. Claim 41 is drawn to the method of claim 40, wherein said probes are selected from a group comprising identification probes. Claims 43, 44, embody claim 40, wherein said probes comprise epithelial specific probes, and tissue specific probes, respectively. Claim 45 is drawn to the method of claim 1, wherein said cell is obtained from a mammal. Claim 46 embodies the method of claim 45, wherein said mammal is a human. Claim 51 specifies the method of claim 1, wherein the cellular marker is an antigen.

Claim 53 is drawn to a method of characterizing a single circulating epithelial cancer cell preparation obtained from a body fluid comprising adhering a cellular preparation comprising said circulating epithelial cancer cell to a surface by means of fixatives, incubating the resulting surface containing fixed cells with multiple fluorescent probes directed to desired cellular markers, wherein said multiple probes have different excitation wavelengths, and wherein said cancer cell preparation is isolated from a body fluid using a negative selection process. Claim 55 embodies the method of claim 53, wherein said single cell is isolated by density gradient centrifugation from a sample containing cells, said isolated cells are fixed onto a surface, said surface containing fixed cells is incubated with said probes , wherein each probe reacts with a marker for the single cell and any probe binding to a marker is examined by a microscope for identification of each individual marker.

Claim 54 is drawn to a method of establishing a characterization profile of a circulating epithelial cancer cell obtained from the body comprising characterizing a single cell environment , by the concurrent measurement of multiple cellular markers using fluorescent probes, wherein said probes emit different wavelengths of light to distinguish multiple cellular markers expressed in said single cell using fluorescence microscopy.

Claims 59 specifies any of the methods of claims 1, 53 and 54, wherein said circulating epithelial cell is a prostatic cancer cell. Claim 62 is drawn to the method of any one of claims 1,

53 and 54, wherein said bodily fluid is selected from the group consisting of blood and body cavity fluid.

Claims 63, 64, 65 and 66 are drawn to the methods of any one of claims 1, 53 and 54 wherein said circulating epithelial cancer cell is obtained from about 5 to 75 ml of blood, 5 to 25 ml of blood and 20 ml of venous blood, respectively. Claims 67, 68 and 69 are drawn to the methods of claims 63, 64 and 65, wherein said circulating epithelial cell is a prostatic cancer cell.

Claim 70 is drawn to any one of claims 1, 53 and 54, wherein said probes are selected from the group consisting of tissue specific probes, probes which bind to tumor cell markers, probes specific for aneuploidy, probes specific for cellular markers of proliferation, probes specific for markers of cell growth inhibition, probes specific for cell cycle arrest, probes specific for cellular markers of apoptosis, and probes specific for hormonal receptors.

Wang et al disclose a method for detecting and characterizing circulating prostatic cancer cells in the blood of a prostatic cancer patient by means of double-density gradient centrifugation of 20 ml of blood; a negative selection process wherein hematopoietic cells are removed by binding to CD45 and glycophorin antibodies which are covalently linked to magnetic beads; the collection of circulating cancer cells after depletion of the hematopoietic cells; a cytospin to deposit the remaining cells comprising cancer cells onto a microscope slide and the subsequent incubation of the microscope slide containing fixed cells with probes (both antibody and nucleic acid) for prostate specific membrane antigen and cytokeratin, a DNA probe for chromosomal centromere 18 and DAPI, a probe for nuclear DNA (introduction, second paragraph, lines 6-9). Wang et al do not specifically disclose that the cells deposited on the microscope by the cytospin procedure were fixed before incubation with the probes, however, the fixation step would be inherent in the method, as unfixed cells would become detached from the slide, and as Wang et al reports the positive identification of prostatic cancer cells, one can reasonably assume that the cells had been fixed to the slide as the cells were present after the step of incubating with the probes. Wang et al do not specifically teach that fluorescent probes were chosen to minimize overlap in the concurrent detection of the cellular markers, but as Wang et al reports that a single circulating epithelial cell is positive for both cytokeratin and aneuploidy of chromosome 18, it can

be concluded that concurrent measurement of emission wavelengths from said single cell differentiated the markers for cytokeratin and the DNA probe for chromosome 18 over the background of a single nucleus stained by DAPI.

6. Claims 1, 11, 12, 13, 14, 16, 17, 37, 38, 40, 41, 45, 46, 60, 62 and 70 are rejected under 35 U.S.C. 102(b) as being anticipated by Shackney et al (Cytometry, 1995, Vol. 22, pp. 282-291, reference AT8 in the IDS filed 4/27/00). The embodiments of claims 1, 11, 12, 37, 38, 40, 41, 45, 46, 60, 62 and 70 are set forth in section 5, above.

Claim 13 embodies the method of claim 1, wherein said fluorescent probes are selected from a group consisting of a mixture of fluorescent probes that emit wavelengths between 400 nm and 850 nm, wherein said emission spectra can be distinguished from each other with the use of a microscope equipped with spectral filters that allow for elimination of overlapping wavelengths. Claim 14 specifies wherein said fluorescent probe emits light with a wavelengths between 430 and 510 nm. Claim 16 specifies the emission of light between 482 and 562 nm. Claim 17 specifies the emission of light with a peak wavelength of about 522 nm.

Shackney et al teach a method to determine aneuploidy in a breast cancer comprising contacting microscope slides comprising cells obtained from a pleural effusion (page 283, second column, under "FISH Studies") with fluorescent probes (FITC) specific for the centromeres of chromosomes 1, 3, 7, 10, 11, 16, 17 and 20. Shackney et al teach each of the probes were individually hybridized with the microscope slides and the nuclear DNA was stained with DAPI for the concurrent visualization of a single centromere probe against the nuclear background to determine the specific number of copies of each of chromosome 1, 3, 7, 10, 11, 16, 17 and 20. A centromere specific probe labeled with FITC and the nuclear DNA probe of DAPI represent two probes and therefore constitute "multiple probes".

7. Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62 and 70 are rejected under 35 U.S.C. 103(a) as being unpatentable over Simpson et al (Experimental Hematology, 1995, Vol. 23, pp. 1062-1068, reference AS9 of the IDS filed 4/27/00) in view of

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human pathology 1996, Vol 25(5)

Waggoner et al (Ref AT15 of the IDS filed 1/28/01) and Galbraith et al (reference AS15 of the IDS filed 1/28/01) and Dale et al (Proc Annu Meet Am Soc Clin Oncol, 1995, Vol. 14, page A1308) and Frudakis et al (6,344,550) and Gross et al (PNAS, 1995, vol. 92, pp. 537-541, cited in a previous Office action).

The embodiments of claims 1, 2, 4, 5, 9, 10, 11, 12, 33-36, 40, 41, 43-46, 51, 54, 56, 62 and 70 are set forth in section 5, above. The embodiments of claims 13, 16, 17 and 60 are set forth in section 6, above.

Claims 18-25 embody specific emission wavelengths of fluorescent probes. Claim 28 embodies the method of claim 13, wherein the fluorescent compounds are selected from a group comprising R-phycoerythrin. Claims 29, 30, 31 and 32 specifically embody the method of claim 1 wherein 4, 5, 6 and 7 fluorescent probes are used concurrently as multiple probes.

Claim 58 embodies the method of claim 4, wherein cells are further isolated by a positive selection process, wherein a specific cell is selected from a heterogenous mixture of cells by an antibody which selectively binds to said specific cell. Claim 60 embodies any one of claims 1, 53 and 54 wherein said circulating epithelial cell is a breast cancer cell.

Simpson et al teach a method for identifying single circulating breast cancer cells in the blood of breast cancer patients comprising density gradient centrifugation on Histopaque 1077 (page 1063, first column, under "Cells and Separation Procedures") followed by a positive selection procedure, wherein an cytokeratin-binding antibody conjugated to magnetic beads enriched the heterologous mixture for the specific cell type. Simpson et al teach the fluorescence labeling of breast epithelial cells and the fluorescent labeling of the contaminating hematopoietic cells by incubation with anti-CK-FITC and anti-CD45-PE. The magnetic beads were labeled with nile red for the enumeration of cell number, thus characterizing the environment of the single cell. Simpson et al teach the selection of nile red beads for their unique fluorescence, thus Simpson et al teach the selection of fluorescent probes based on minimum overlap of emission spectra. Simpson et al teach visualization of the labeled cells by flow cytometry and the deposition of labeled cells, sorted by flow cytometry, onto a microscope slide (page 1064, first column, first full

paragraph) for further visualization. Simpson et al do not teach the labeling of the cells with fluorescent probes while attached to the slide.

The abstract of Dale et al (Proc Annu Meet Am Soc Clin Oncol, 1995, Vol. 14, page A1308) teaches the improvement in sensitivity and specificity attained by assaying for circulating melanoma cells using multiple markers in place of single markers.

Frudakis et al (6,344,550) teach multiple markers for detecting breast cancer cells.

Gross et al (PNAS, 1995, vol. 92, pp. 537-541, cited in a previous Office action) teaches a model study for the detection of breast cancer cells in peripheral blood comprising the use of three different anticytokeratin antibodies labeled with PerCP, PE and APC, respectively, and antibodies directed against hematopoietic cells (anti-CD45, anti-CD42a, anti-CD61, andi-CD34 and anti-glycophorin) labeled with FITC. Gross et al teach the concurrent measurement of the three probes in a single breast cancer epithelial cell by means of flow cytometry.

Galbraith et al teach that fluorescent imaging cytometry is superior to flow cytometry in that the method is not limited by the number of fluorophores which may be distinguished (page 592, first column second full paragraph). Galbraith et al teach the selection of flurophores that have spectral properties which are sufficiently separated as to be measured independently of one another and the optimization of the excitation and emission filters in the microscope (page 562, first column, third full paragraph).

Waggoner et al teach multicolor analysis of populations of single cells and a microscope slide surface containing cells (page 499, first column, "Multicolor Immunophenotyping"), wherein the cells were well separated from each other by quantitative fluorescent imaging (page 499, second column, last paragraph). Waggoner et al teach spectroscopic properties of selected probes (Table 1, including fluorescent haptens, reagents for determining DNA/RNA content, and probes for membrane location and fluidity, and physiological indicator probes sensitive to intracellular calcium concentration and pH).

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to fix the density gradient separated cells, as taught by Simpson et al, onto a microscope slide and incubate with multiple fluorescent probes specific to breast cancer.

One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Dale et al on the increased sensitivity and specificity afforded by detection of multiple markers versus single markers for the detection of circulating melanoma cells; the teachings of Frudikas on breast tumor specific antigens (Table I); the teachings of Gross et al on the improvements of sensitivity and specificity afforded by the detection of breast cancer cells seeded into peripheral blood which are labeled with multiple probes consisting of three different cytokeratin antibodies (abstract); the teachings of Galbraith et al on the selection of fluorescent probes which have non-overlapping spectra and the teachings of Waggoner et al on the available fluorescent probes, and the desirability of a microscope slide preparation of cells for the quantification of cellular fluorescence after incubation with multiple fluorescent probes.

8. Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, 70 and claims 47, 49, 50 and 52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Simpson et al (Experimental Hematology, 1995, Vol. 23, pp. 1062-1068, reference AS9 of the IDS filed 4/27/00) in view of Waggoner et al (Ref AT15 of the IDS) and Galbraith et al (reference AS15 of the IDS) and Dale et al (Proc Annu Meet Am Soc Clin Oncol, 1995, Vol. 14, page A1308) and Frudakis et al (6,344,550) and Gross et al (PNAS, 1995, vol. 92, pp. 537-541, cited in a previous Office action) as applied to claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62 and 70 above, and further in view of the abstract of Zhang et al (Chinese Journal of Surgery, 1997, Vol. 35, pp. 474-477) and the abstract of Kute et al (Cytometry, 1983, Vol. 4, pp. 132-140).

Simpson et al and Waggoner et al, and Galbraith et al, and Dale et al, and Frudkis et al and Gross et al teach the embodiments of claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62 and 70, for the reasons set forth in section 7, above.

Claim 47 embodies the method of claim 40 wherein said probes are used to detect a hormone receptor or hormone receptor gene. Claims 49 and 50 specifically embody the estrogen receptor and the progesterone receptor, respectively. Claim 52 embodies the cellular antigen of claim 51, wherein the antigen is a receptor. Neither Simpson et al nor Waggoner et al, nor

Galbraith et al nor Dale et al nor Frudkis et al nor Gross et al teach fluorescent imaging cytometry of circulating epithelial cells by means of fluorescent probes which bind to the estrogen or progesterone receptors.

Kute et al teach cytofluorometric analysis for estrogen receptors using fluorescent estrogen probes. Kute et al teach the analysis by flow cytometry. Zhang et al teach the prognosis of node-negative breast cancer patients by determination of the estrogen receptor or progesterone receptor status of tumor tissues.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to include fluorescent probes for the estrogen or progesterone receptors in the imaging cytometry of metastatic breast cancer cells as taught by Simpson et al and Waggoner et al, and Galbraith et al, and Dale et al, and Frudkis et al and Gross et al.

One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Zhang et al on the prognostic importance of estrogen and progesterone receptor status in breast cancer patients, and the teaching of Kute et al on the correlation between estrogen receptor positive status and a response to hormonal therapy.

9. Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, 70 and 59 are rejected under 35 U.S.C. 103(a) as being unpatentable over Simpson et al (Experimental Hematology, 1995, Vol. 23, pp. 1062-1068, reference AS9 of the IDS filed 4/27/00) and Waggoner et al (Ref AT15 of the IDS filed 1/28/01) and Galbraith et al (reference AS15 of the IDS filed 1/28/01) and Dale et al (Proc Annu Meet Am Soc Clin Oncol, 1995, Vol. 14, page A1308) and Frudakis et al (6,344,550) and Gross et al (PNAS, 1995, vol. 92, pp. 537-541, cited in a previous Office action) as applied to claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62 and 70 above, and further in view of Ferrari et al (Proc Annu Meet Am Assoc Cancer Res, 1996, vol. 37, pp. A1686).

Simpson et al and Waggoner et al, and Galbraith et al, and Dale et al, and Frudkis et al and Gross et al teach the embodiments of claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62 and 70, for the reasons set forth in section 7, above. Simpson et al and

Waggoner et al, and Galbraith et al, and Dale et al, and Frudkis et al and Gross et al do not teach the detection of a circulating epithelial cell which is prostatic cancer.

Ferrari et al teach the detection of circulating metastatic prostate tumor cells by PCR amplification of the PSM prostate specific integral membrane antigen. Ferrari et al teach that the detection of these cells is useful in identifying subgroups of advanced prostate cancer patients.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to detect circulating prostatic cancer cells in the blood of men with prostate cancer by multiple fluorescent probes and fluorescent imaging cytometry.

One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Gross et al on the detection of targeted cancer cells in peripheral blood at frequencies as low as 1 in 10^7 by flow cytometry, the teachings of Ferrari et al on the detection, by PCR, of prostate cancer cells in peripheral blood at frequencies as low as 1 in 10^6 and the teaachings of Simpson on the similar results obtained from fluorescent imaging cytometry versus flow cytometry.

10. Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, 70, 59 and claims 47, 48 and 52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Simpson et al (Experimental Hematology, 1995, Vol. 23, pp. 1062-1068, reference AS9 of the IDS filed 4/27/00) in view of Waggoner et al (Ref AT15 of the IDS filed 1/26/01) and Galbraith et al (reference AS15 of the IDS) and Dale et al (Proc Annu Meet Am Soc Clin Oncol, 1995, Vol. 14, page A1308) and Frudakis et al (6,344,550) and Gross et al (PNAS, 1995, vol. 92, pp. 537-541, cited in a previous Office action) and Ferrari et al (Proc Annu Meet Am Assoc Cancer Res, 1996, vol. 37, pp. A1686) as applied to claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62 and 70 above, and further in view of the abstract of Takeda et al (Cancer, 1996, Vol. 77, pp. 934-940).

Simpson et al and Waggoner et al, and Galbraith et al, and Dale et al, and Frudkis et al and Gross et al, and Ferrari et al teach the embodiments of claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62 and 70 for the reasons set forth in section 9, above.

Claim 47 embodies the method of claim 40 wherein said probes are used to detect a hormone receptor or hormone receptor gene. Claim 48 embodies the androgen receptor. Claim 52 embodies the cellular antigen of claim 51, wherein the antigen is a receptor. Neither Simpson et al nor Waggoner et al, nor Galbraith et al nor Dale et al nor Frudkis et al nor Gross et al nor Ferrari et al teach fluorescent imaging cytometry of circulating epithelial cells by means of fluorescent probes which bind to the androgen receptor.

Takeda et al teach the immunohisotchemical detection of the androgen receptor in patients with prostate carcinoma.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to include fluorescent probes for the androgen receptor in the imaging cytometry of metastatic prostate cells as taught by Simpson et al and Waggoner et al, and Galbraith et al, and Dale et al, and Frudkis et al and Gross et al and Ferrari et al.

One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Takeda et al on the prognostic importance of androgen receptor status in prostate cancer patients.

11. Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, 70 and claims 53 and 55 are rejected under 35 U.S.C. 103(a) as being unpatentable over Simpson et al (Experimental Hematology, 1995, Vol. 23, pp. 1062-1068, reference AS9 of the IDS filed 4/27/00) in view of Waggoner et al (Ref AT15 of the IDS filed 1/28/01) and Galbraith et al (reference AS15 of the IDS filed 1/28/01) and Dale et al (Proc Annu Meet Am Soc Clin Oncol, 1995, Vol. 14, page A1308) and Frudakis et al (6,344,550) and Gross et al (PNAS, 1995, vol. 92, pp. 537-541, cited in a previous Office action) in view of Thomas et al (US 6,117,985).

Simpson et al and Waggoner et al, and Galbraith et al, and Dale et al, and Frudkis et al and Gross et al teach the embodiments of claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62 and 70, for the reasons set forth in section 7, above.

Claim 53 is drawn to a method of characterizing a single circulating epithelial cancer cell preparation obtained from a body fluid comprising adhering a cellular preparation comprising said

circulating epithelial cancer cell to a surface by means of fixatives, incubating the resulting surface containing fixed cells with multiple fluorescent probes directed to desired cellular markers, wherein said multiple probes having different excitation wavelengths, and wherein said cancer cell preparation is isolated from a body fluid using a negative selection process. Claim 55 embodies the method of claim 53, wherein said single cell is isolated by density gradient centrifugation from a sample containing cells, said isolated cells are fixed onto a surface, said surface containing fixed cells is incubated with said probes , wherein each probe reacts with a marker for the single cell and any probe binding to a marker is examined by a microscope for identification of each individual marker.

Simpson et al and Waggoner et al, and Galbraith et al, and Dale et al, and Frudkis et al and Gross et al teach the embodiments of claims 53 and 55 with the exception of the negative selection process.

Thomas et al teach a process for enriching non-hematopoietic tumor cells from peripheral blood based on negative selection. Thomas et al teach that the method specifically embodies the enrichment of epithelial cancers of the bronchi, mammary ducts, gastrointestinal tract, and carcinomas of the lung, breast, colon, prostate, bladder, ovary, pancreas, and rectum.

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to substitute the negative selection process as taught by Thomas et al for the sorting by flow cytometry as taught by Simpson et al.

One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Thomas et al on the efficacy of enriching tumor cells from peripheral blood by a negative selection based on the use of antibody cocktails which bind hematopoietic cells.

12. Claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62, 70 and 61 are rejected under 35 U.S.C. 103(a) as being unpatentable over Simpson et al (Experimental Hematology, 1995, Vol. 23, pp. 1062-1068, reference AS9 of the IDS filed 4/27/00) in view of Waggoner et al (Ref AT15 of the IDS filed 1/28/01) and Galbraith et al (reference AS15 of the

IDS filed 1/28/01) and Dale et al (Proc Annu Meet Am Soc Clin Oncol, 1995, Vol. 14, page A1308) and Frudakis et al (6,344,550) and Gross et al (PNAS, 1995, vol. 92, pp. 537-541, cited in a previous Office action) in view of the abstract of Z'Graggen et al Pancreas, 1997, Vol. 15, No.4, page 463) or the abstract of Leather et al (British Journal of surgery, 1993, vol. 80, pp. 777-780) or the abstract of Komeda et al (Cancer, 1995, vol. 75, pp. 2214-2219).

Claim 61 embodies the method of any one of claims 1, 53 and 54 wherein said circulating epithelial cell is selected from the group consisting of liver, kidney, colon, rectum, gastric, esophageal, bladder, brain, ovary, pancreas and lung cancer cells.

Simpson et al and Waggoner et al, and Galbraith et al, and Dale et al, and Frudkis et al and Gross et al teach the embodiments of claims 1, 2, 4, 5, 9, 10-13, 16-25, 28-36, 40, 41, 43-46, 51, 54, 56, 58, 60, 62 and 70, for the reasons set forth in section 7, above. Simpson et al and Waggoner et al, and Galbraith et al, and Dale et al, and Frudkis et al and Gross et al do not teach the detection of a circulating epithelial cell which consist of liver cancer cells, colorectal cancer cells or pancreatic cancer cells.

Z'Graggen et al teach that circulating pancreatic tumor cells correlates to the presences of distant metastasis in pancreatic cancer patients.

Leather et al teach the detection and enumeration of circulating tumor cells in colorectal cancer to be indicative of the metastatic process.

Komeda et al teach the detection of circulating hepatocellular carcinoma cells in peripheral venous blood of patients is indicative of the metastatic process

It would have been *prima facia* obvious to one of ordinary skill in the art at the time the claimed invention was made to detect circulating liver, pancreatic or colorectal cancer cells in the blood of patients with liver, pancreatic or colorectal cancer by multiple fluorescent probes and fluorescent imaging cytometry.

One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Simpson et al, and Gross et al and Dale et al and Waggoner et al and Galbraith et al on the efficacy of detecting a target epithelial tumor cell type from the blood of individual afflicted with said target tumor cells; and the teachings of Z'Graggen

et al, Leather et al and Komeda et al on the correlation between the detection of the target tumor cells in the peripheral blood, and the presence of metastatic disease.

13. All other rejections and objections as statd in Paper No. 6 are withdrawn due to applicants amendments to the claims.

Conclusion

14. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Karen Canella whose telephone number is (703) 308-8362. The examiner can normally be reached on Monday through Friday from 8:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anthony Caputa, can be reached on (703) 308-3995. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

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